SUPPLEMENTAL RESPONSE TO RESTRICTION REQUIREMENT

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Title: siRNA EXPRESSION SYSTEM

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IN THE CLAIMS

- 1. (Original) A recombinant vector for the correct, stable and effective expression in mammalian cells of a siRNA or a miRNA, comprising from 5' to 3':
- a) an RNA polymerase II dependent promoter sequence derived from the U1 snRNA gene;
- b) suitable restriction sites for cloning the sequence that transcribes a pre-siRNA or a pre-miRNA;
- c) a sequence transcribing the pre-siRNA comprising: in position +1 an A or a G residue; a sequence from 21 to 23 nucleotides corresponding to a sense region of the mRNA transcribed by the gene to be silenced, that constitutes the first segment of the stem of the pre-siRNA; a sequence selected from a pre-miRNA sequence that constitutes the loop region of the pre-siRNA; a sequence from 21 to 23 nucleotides corresponding to the antisense region of the mRNA transcribed by the gene to be silenced that constitutes the second segment of the stem of the pre-siRNA; two final residues UU protruding in such a way that the following structure is obtained:

or alternatively a sequence transcribing the pre-miRNA;

- d) termination sequences derived from the sequence at 3' of the gene for U1 snRNA which are necessary and sufficient for the correct formation of the 3' of the pre-siRNA or of the pre-miRNA.
- 2. (Original) The vector according to Claim 1, wherein the cloning site for the 5' of the sequence transcribing the pre-siRNA is Bgl II.
- 3. (Original) The vector according to Claim 1, wherein the sequence transcribing the pre-siRNA further comprises at termini 5' and 3' such sequences that the transcribed pre-siRNA has

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the following structure:

where N is A, U, G or C and N' is its complementary nucleotide.

4. (Original) The vector according to Claim 3, wherein the sequence transcribing the presiRNA comprises at the 5' and 3' termini such sequences that the transcribed pre-siRNA has the following structure:

- 5. (Original) The vector according to claim 4 wherein the termination sequences derived from the sequence at 3' of the gene for U1 snRNA are as follows:

 CCCCTG/ACTTTCTGGAGTTTCAAAAGTAGAC.
- 6. (Original) Vector according any of previous claims further comprising suitable sequences to make inducible the RNA pol II promoter.
- 7. (Original) A composition for gene therapy comprising the vector according to any of previous claims.
- 8. (Canceled)